Testimony
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Assessing Anthrax Detection Methods

Statement of

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Good afternoon, Chairman Shays and members of the Subcommittee. I am Dr. Tanja Popovic, Acting Associate Director for Science with the Centers for Disease Control and Prevention (CDC). Accompanying me today is Mr. Max Kiefer, Assistant Director for Emergency Preparedness and Response for CDC's National Institute for Occupational Safety and Health (NIOSH). On behalf of CDC, I am pleased to provide this testimony describing our views on validation issues, our work with the United States Postal Service (USPS) detecting anthrax contamination during the bio-terrorism attacks of 2001, and ongoing research and developments to improve environmental testing methods.

CDC is part of the Department of Health and Human Services (HHS). As the nation's disease prevention and control agency, CDC's responsibility is to provide national leadership in the public health and medical communities in a concerted effort to detect, diagnose, respond to, and prevent injury and illnesses, including those that occur as a result of a deliberate release of biological agents.

During the anthrax attacks of 2001, CDC assumed a wide range of responsibilities including surveillance to detect new cases of illness; epidemiologic investigations to assess the risks of infection; collection of environmental samples to determine the extent of contamination in affected buildings; analysis of environmental and clinical laboratory specimens; delivery of stockpiled antibiotics and vaccine; follow-up of persons receiving stockpile

products; and communication with the public and with public health professionals to provide up-to-date guidance and recommendations.

Once the emergency phase was complete, CDC published peer-reviewed reports on each of the outbreak investigations to share findings and improve scientific understanding of bio-terrorism incidents. CDC initiated research to improve our environmental sampling tools and provided technical advice on environmental sampling and related issues to the Environmental Protection Agency (EPA) and others working to remediate and restore anthrax-contaminated buildings.

Since 2001, CDC has collaborated with other agencies on several new efforts. For example, CDC worked with the Departments of Homeland Security and Justice and the Environmental Protection Agency in developing the draft BioWatch Preparedness and Response Guidance, a three part tool that provides guidance for preparedness, response and environmental sampling as it pertains to this environmental surveillance effort initiated by the Department of Homeland Security (DHS). This draft guidance was created in collaboration with, and has been approved by HHS, DHS, Department of Justice (DOJ), and EPA. CDC is working with DHS and national laboratories on issues related to restoration of public transportation systems in the event of a bio-terrorist act. In sum, CDC has worked to improve preparedness and prevention capabilities and has worked with other government agencies to enhance coordination and fill research gaps.

The invitation letter from the Subcommittee asked CDC to address a number of technical environmental sampling issues. Our comments on these various issues are provided below.

Environmental sampling

Environmental testing of potentially contaminated facilities played an important role in the 2001 CDC response. Subject matter experts at CDC guided efforts to collect and analyze surface, bulk, and air samples for *Bacillus anthracis*. CDC consulted with military and other experts and revised guidance for conducting environmental sampling and lab analysis based on the best available information and incorporation of ongoing experience. Existing programs, such as the Laboratory Response Network (LRN), which links state and local public health laboratories with advanced capacity laboratories, were strengthened in the enormous effort to enlist resources to identify potential contamination. During the anthrax attacks, LRN laboratories tested more than 125,000 environmental specimens alone, which represented over 1 million individual laboratory tests.

Environmental sampling helped identify the likely source of infection and exclude alternative sources. It improved CDC's understanding of environmental exposure pathways, including the potential for re-aerosolization of spores and informed decisionmaking about chemoprophylaxis of exposed individuals, remediation, and re-occupancy.

The concept of environmental sampling has expanded since 2001 to include the development of early warning systems to provide a signal should a new event occur. These new systems rely on a phased approach. Detection of suspect bio-agents via ongoing sample collection and analysis is the first phase. Biowatch or the USPS "Biohazard Detection System (BDS)" are examples of such systems. Identification is the second phase. It involves confirmation via high-reliability LRN laboratory testing to confirm and identify the presence of a bio-agent. Additional sampling by law enforcement and public health representatives may occur during the investigation/response phase to further guide interventions. These systems work in parallel with the national health monitoring initiative known as "BioSense" which monitors illness trends to provide additional early detection capability to our national public health system.

Validation

The term validation is used by environmental sampling experts to describe quality assurance testing needed to determine the reliability of a given method. It generally involves comparing the performance of a method against either a "reference method" or a known concentration to establish the precision, accuracy, and the upper and lower limits of the method. Environmental testing generally involves at least a sample collection step and a sample analysis step to identify the presence of an agent and, if possible, an estimate of the amount present. No method allows 100% recovery of an agent. Validation is often done

in steps and methods that show promise after early testing may receive more testing to address issues such as inter-lab variability.

At the time of the 2001 bio-terrorism events, the LRN sample analysis protocols used to identify *B. anthracis* had been validated, and member labs participated in a proficiency testing program. These LRN protocols were used for analysis of both clinical and environmental samples and had been validated for processing and detecting *B. anthracis* spores. In addition, evidence demonstrated that the spores would most likely not be affected by various shipment methods. In 2001, data were available to suggest that light and temperatures encountered in transport would not have negatively affected anthrax spore viability.

However, validated methods for sample collection of *B. anthracis* via surface or air sampling did not exist at that time. Because of this, CDC used caution when interpreting results from these methods, and CDC included explicit messages about the lack of validation on all sampling guidance used internally and shared with other governmental partners.

While the available sample collection methods lacked validation for *B. anthracis*, established methods had been validated for sampling of other biological agents. Existing methods were extrapolated to the collection of anthrax spores in consultation with subject matter experts.

CDC began planning for quality assurance testing for these methods shortly after the emergency response phase ended. Validation for biological agents is complicated because it involves dealing with living systems. Not all cells will respond or grow given the same nutrients, humidity, and temperature conditions. Reliably creating known concentrations of spores on surfaces and in air for validation studies is a time-consuming technical challenge. In addition, validation for bio-terrorism agents is especially challenging as there are limited facilities to do such studies. As a result of these complexities, it would not have been technically possible for CDC to validate sampling methods during the anthrax attack response in 2001.

CDC partnered with USPS in a research project to utilize existing contaminated surfaces at postal facilities for comparing the collection capabilities of surface sampling methods. These "side-by-side" tests performed in December 2001, sought to overcome the time consuming technical challenges associated with generating known surface contamination levels in a laboratory. The side-by-side tests scientifically compared each of the key methods to other methods and provided an important objective basis for method selection. While this research did not equal "comprehensive" validation, it directly addressed the most important data gaps associated with method selection. Similar testing involving air sampling was performed at the USPS Trenton facility shortly after the public health emergency in February 2002.

Detecting anthrax contamination in Postal facilities

CDC performed outbreak investigations in Florida, New York, New Jersey, Washington, D.C., and Connecticut in response to disease cases resulting from the anthrax attacks. Each investigation involved a multi-disciplinary public health team that included CDC and local and state health department representatives. Teams coordinated closely with USPS and law enforcement representatives. Environmental sampling was integrated into each outbreak investigation. The purpose of these investigations was to identify the source of exposure and to determine if additional public health interventions were needed (e.g. antibiotic prophylaxis, vaccines, facility closures). Outbreak-related sampling was performed in three types of settings: 1) facilities where postal employees at the facility contracted anthrax (e.g. Trenton and Brentwood); 2) facilities that were part of epidemiologic investigations looking for clues on the role that cross-contaminated mail may have played in non-postal employee cases; and 3) facilities where sampling, epidemiologic, or mail flow patterns suggested cross-contamination of mail may have resulted in their contamination (e.g. all 50 post offices upstream/downstream of the Trenton sorting facility). CDC tested 112 facilities and found 12 facilities with positive results. Another seven facilities in Florida were sampled in collaboration with the Environmental Protection Agency (EPA) – all seven involved positive results. Surface testing involved methods such as swabs, wipes, and vacuum sock samples.

Sampling strategies

CDC used targeted (also known as epidemiologically driven) sampling strategies during the outbreak investigations to determine where to collect environmental samples. Incident-specific details (epidemiologic data, interviews with USPS personnel, and understanding of the mail handling process) were used to help identify locations considered most likely to be contaminated so that environmental samples could be collected at these locations. This "worst case" approach used well-accepted empirical approaches to identify plausible contamination pathways. The primary objective in most cases was to maximize the possibility of finding contamination. For example, targeted sampling utilized postal code information printed on the recovered Daschle letter envelope to identify that Brentwood Delivery Bar Code Sorter (DBCS) #17 had processed the letter. Machine #17 was sampled and specific attention was given to locations closest to #17's mail path.

CDC believes that a targeted sampling strategy is the most rapid, efficient, and successful approach when information is available on the path of the terrorism source or vehicle. Targeted strategies not only produced the highest probability of identifying a positive sample during the 2001 response, but also helped to establish locations that posed the greatest risk of exposure.

Targeted sampling must be supplemented with other approaches when there is a lack of incident information to direct samples. Full inspection approaches, where 100 percent of a type of surface is targeted may also be needed. In addition,

CDC believes there is a need to further develop probabilistic sampling approaches (i.e. using random sampling and statistical inferences) to provide additional sampling strategy tools.

Evaluating the meaning of environmental sampling results

Several factors fostered CDC's confidence in interpreting and evaluating environmental sampling results during and after the 2001 events. The methods led rapidly to successful collection of positive anthrax samples during the investigations. Experienced industrial hygienists trained to recognize and evaluate complex hazardous environments were used to perform the sampling. Routine advance communication between the industrial hygienists collecting the samples and the LRN Level B laboratory experts analyzing the samples ensured mutual understanding on sampling methods, sample numbers, shipment, and analysis. In the absence of validated methods specifically for anthrax, these factors and this teamwork model strengthened CDC's confidence in the results obtained from testing.

Furthermore, CDC used care in evaluating the meaning of sample results. CDC understood that results would be "qualitative" (positive/negative) and that there were no available health based criteria for evaluating environmental contamination levels. As a result, no formal criteria were used to further distinguish among positive results. CDC also understood that air sample results

collected some time after facility closure might be negative and that surface samples were more reliable indicators of contamination after the fact.

CDC emphasizes that environmental sampling information was not used in isolation but in conjunction with other outbreak investigation information such as epidemiology findings and facility engineering and work practice information. At times, interventions were recommended based on the larger picture despite a lack of positive environmental results. Care was used in interpretation of "negative" test results given the recognition that the lowest limit of detection (i.e. the minimum concentration of anthrax spores that can be detected) for the methods was not available. For example, local illness surveillance activities were continued to provide alternative sources of information.

Based on GAO's recommendation during the May 2003 Congressional hearings, CDC also worked with USPS and other government agencies and the postal worker unions to evaluate whether additional environmental sampling was warranted at facilities that had tested negative based on earlier sampling results. This led to a report issued on August 27, 2004, that concluded that the anthrax risk level for postal workers in the facilities tested, along with the general public served by those facilities was negligible and that no further sampling was warranted. Key factors in the conclusion were the use of anthrax-related cleaning efforts, controls, and work practices at USPS facilities; the nature of sampling performed at facilities known to have processed the source letters; and

the passage of two and a half years without additional health concerns (USPS, 2004).

CDC also worked with USPS and public health partners to create guidance for responding to detection of aerosolized *B. anthracis* by Autonomous Detection Systems in the workplace. This guidance, published in CDC's *Morbidity Mortality Weekly Report* in April 2004, describes the arrangements needed to confirm positive signals and how to develop effective response protocols for such signals. The guidance was designed to meet the needs of USPS for their BDS system and to provide a template for use by other organizations deciding to deploy such systems (Meehan, et al., 2004).

Efforts toward improving and validating sampling protocols

CDC believes that full validation of every possible scenario variation would be impractical and could not take the place of scientific judgment and evaluation of the specific event. However, CDC is making efforts to validate components of the detection process.

Comparative studies

As previously described, CDC conducted comparative "side-by-side" studies at the Brentwood (now Curseen/Morris) postal facility to compare the effectiveness of different surface sampling methods for detecting anthrax spores. The studies compared performance of dry swabs, wet swabs, wet wipes, and vacuum

methods. The applied research also examined the performance of Polymerase Chain Reaction (PCR) technology in comparison to culture approaches. At the Trenton postal facility, CDC performed "side by side" testing to evaluate the sensitivity of several air sampling methods and filter types. These studies provided important information on the performance of the methods. Results were shared with USPS, EPA, and other investigators and were published in the peer reviewed literature to improve overall assessment capabilities (Sanderson, et al, 2002). This information was utilized at facilities undergoing remediation for characterization and clearance sampling.

Laboratory studies

Since 2001, CDC has learned that sampling materials are different, both in their ability to remove spores from surfaces or to release them during sample processing. For swabs, the two materials with the best recovery are cotton tipped swabs or macrofoam swabs (Rose LJ, et al, 2004). In addition, CDC has confirmed what has been historically known about surface sampling with swabs: pre-moistened swabs work better. Because of the uncontrolled variables in the sampling process the limit of detection may be a range and not an absolute number. Additional study is needed as these results are based on studies of a single laboratory, and validation will require multiple laboratory participation.

LRN enhancements

CDC acknowledges that laboratory analytic capacity was stretched during the 2001 events, especially in regard to laboratories in closest proximity to the unfolding events. Since then, a strategy for the transport and shipping of sample burdens to other competent LRN labs that are distal to the most heavily impacted labs has been formulated. The capacity to perform real-time PCR, especially at the LRN confirmatory Reference level (formerly known as LRN levels B and C laboratories), has also increased dramatically since 2001. Should another bio-terror event occur, the LRN will mobilize a phone bank of LRN representatives to retrieve periodic updates of laboratory capacity and projected sample throughput. In addition, the LRN has made advances in electronic data exchange in order to facilitate the rapid communication of laboratory test results in an emergency situation. .

Collaborative studies

Dugway

Via a September 2002 interagency agreement with the U.S. Army's Dugway Proving Grounds in Utah, CDC is supporting research to improve environmental exposure sampling methods for bio-terrorism response investigations. The study uses three surface concentrations and three air concentrations. These concentrations are expected to allow for estimates of lower limits of detection for the sampling methods. The study will also generate samples for additional EPA testing of QPCR (Quantitative Polymerase Chain Reaction) at an offsite facility.

Lastly, the Dugway test chamber and protocol will support testing of additional agents or their non-pathogenic simulants. Preliminary work was recently completed and testing is now underway to:

a) Determine the efficiency of three surface sampling methods (wet swab, wet wipe, and surface vacuum filter sampling) on two types of surfaces (stainless steel and carpet);
b) Determine the efficiency of three air sampling methods (Andersen single stage impactor, PTFE filters, and gel filters);
c) Determine the overall precision of the methods, encompassing sample collection, sample extraction, and sample analysis;
d) Determine intra-lab variability and sample transport factors; and
e) Determine the additional sampling collection efficiency of passing over a surface multiple times.

Sandia

CDC is collaborating with the Sandia National Laboratories in New Mexico and EPA on a DHS funded effort to evaluate current surface sample and extraction methods. The study will also allow for estimates of the lower limits of detection for the methods. Testing is underway to:

a) Determine the efficiency of three surface sampling methods (wet swab, wet wipe, and surface vacuum filter sampling) on four types of surfaces (2 non-porous - stainless steel and painted wallboard; and 2 porous – carpet and bare concrete);

b) Determine the overall collection efficiency of the methods, encompassing sample collection, sample extraction, and sample analysis; and
c) Determine if collection efficiencies are a function of the concentration of spores on the surface being tested.

Summary

Environmental microbiology and sampling issues are important – they provide tools needed for public health decisions, law enforcement investigations, and evaluation of remediation success. CDC is sponsoring research to fill a number of validation gaps for *B. anthracis*. There are many bio-agents of interest, and interagency collaboration via DHS coordination is important for moving forward to improve our overall methods. CDC plans to review the upcoming GAO report closely, and we will work with DHS, EPA, and other agencies to further address issues identified in the report.

This concludes my testimony. I will be happy to answer any questions.

References

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